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Previews

Synaptobrevin, Sphingolipids, and Secretion: Lube 'n' Go at the Synapse

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For neurotransmitter release to occur, proteins and lipids have to work together. The classical view of this process is that a variety of proteins work hard to force the unwilling, fusion-aversive lipids into merging. In this issue of *Neuron*, a study by Darios et al. paints the opposite picture: a lipid metabolite stimulates the reluctant vSNARE synaptobrevin to engage in fusogenic protein complexes.

Biological membranes are heterogeneous and complex mosaics of lipid and protein domains that accumulate specific cellular signaling components. The correct organization and location of these domains provides the right environment for proteins of the secretion machinery to operate effectively in docking, priming, and fusion of different secretory vesicles, such as synaptic vesicles at the presynaptic active zone and large dense-cored vesicles in neurons and neuroendocrine cells.

Probably the best described example of the permissive role that lipids play in secretion is the regulated turnover of phosphoinositides, especially the PtdIns(4,5)P2 pathway (for a review see Rohrbough and Broadie, 2005). Such lipids in the target membrane (and probably also in the vesicle membrane) interact with important components of the secretion machinery like synaptotagmins, DOC2, rabphilin, and CAPS. Furthermore, one PtdIns(4,5)P2 metabolite, diacylglycerol, is a potent enhancer of presynaptic secretion by activating several interdependent signaling cascades in the presynaptic terminal (Wierda et al., 2007). Finally, cholesterol in brain membranes is required for efficient fusion, by the accumulation of components of the secretion machinery, like syntaxin, in cholesterol-rich domains in the plasma membrane (Lang et al., 2001).

In this issue of *Neuron*, Darios et al. (2009) provide evidence for a rather specific stimulatory role of a different type of lipid: sphingosine. Sphingosine is a positively charged 18 carbon amino alcohol with a trans-unsaturated hydrocarbon chain and a minor constituent in most biological membranes. This lipid is derived from one of the five common phospholipids, sphingomyelin, via two enzymatic reactions (Figure 1) involving ceramide as an intermediate. Sphingolipids self-aggregate into membrane domains together with cholesterol (see Rohrbough and Broadie [2005] for a review). Both ceramide and sphingosine rapidly flip between both sides of the bilayer at neutral pH, and especially sphingosine readily partitions between membranes across the water phase. Darios et al. now show that sphingosine exerts a potent positive effect on the formation of SNARE complexes in vitro and on secretion in a variety of cellular assays: synaptosomes, hippocampal synapses, neuroendocrine cells, and the neuromuscular junction. This effect is specific for one category of biological lipids and also appears to be specific in terms of its target, since sphingosine no longer potentiates secretion in synaptobrevin-2 null mutant neurons. This is a remarkable finding given the fact that addition of exogenous sphingosine may induce a broad spectrum of nonspecific changes, for instance in membrane fluidity and curvature and the distribution of membrane proteins. The authors propose that sphingosine, being positively charged, exerts its actions by locally disrupting electrostatic and hydrophobic interactions of the cytosolic part of synaptobrevin with negatively charged phosphatidylserine in vesicular membranes. Interestingly, the stimulatory effect appears to be based, at least largely, on an increase in the amount of vesicles available for release (readily releasable pool, as assayed by application of hypertonic sucrose). This suggests that the availability of synaptobrevin is rate limiting during an upstream step in the secretory pathway (docking/priming).

These important and well-documented findings provide an important link with two previous, underexposed findings in invertebrates. First, secretion defects in yeast cells deficient for Snc, a yeast ortholog of synaptobrevin, were rescued by Vbm/Elo, two enzymes that are involved in long-chain fatty acid elongation and are responsible for intracellular accumulation of phytosphingosine (David et al., 1998). This rescue effect is exerted via activation of a sphingoid base/ceramide-activated phosphatase, Sit4, and dephosphorylation of its target tSNARE Sso2, a yeast ortholog of syntaxin. Second, mutations in the gene encoding a fly ceramidase, slug-a-bed (Slab), produce secretion defects in the fly neuromuscular junction (Rohrbough et al., 2004). The observation that fluorescently tagged ceramide (or its metabolites) accumulated in synapses confirmed the ample presence of sphingolipids in the presynaptic membrane and synaptic vesicles (see also Takamori et al., 2006).

Together with these two indications from genetic studies, the findings by Darios et al. clearly support an evolutionary conserved role of sphingolipid metabolism in secretion. Two possible scenarios can be envisioned to reconcile all currently available data (Figure 1): sphingosine readily flips between two

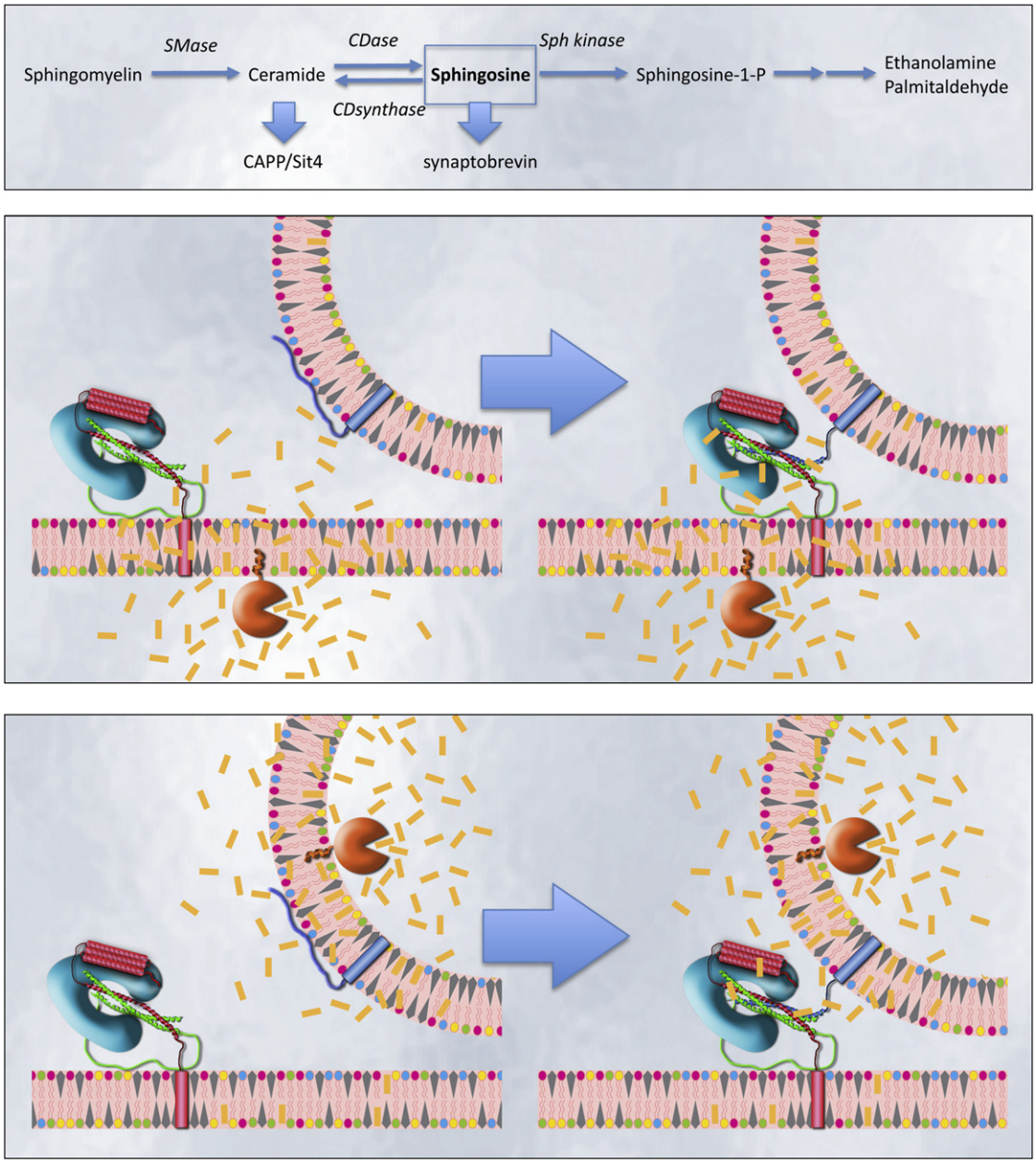

Figure 1. Cartoon Depicting the Main Pathway in Sphingosine Metabolism and Two Alternative Possibilities to Locally Generate Sphingosine for Synaptobrevin Activation

SMase, sphingomyelinase; CDase, ceramidase; CDsynthase, ceramide synthase; Sph kinase, sphingosine kinase; CAPP, ceramide-activated protein phosphatase. Products on the far right are the final breakdown products after multiple enzymatic steps. Cartoon depicts plasma and vesicle membrane with different phospholipids (different color circles), cholesterol (gray cones), and sphingosine (orange sticks) and two possible local sources of sphingosine: originating from ceramidase activity (orange structures) located either on the outside of the plasma membrane (top row) or the inside of the synaptic vesicle (bottom row). Local sphingosine production (cloud of orange sticks emerging from the ceramidase) is proposed to promote association of vesicular, unstructured synaptobrevin (blue structure) into a four helical bundle that also contains syntaxin (red) and SNAP-25 (green) and that is promoted by Munc18 (blue horseshoe).

membrane leaflets, but it becomes trapped in the inner leaflet of the vesicle membrane because it contains a positive charge due to the acidic luminal pH of the secretory vesicle, which will prevent spontaneous flip to the cytosolic leaflet. This will leave the basal sphingosine concentration in the outer leaflet submaximal for synaptobrevin's engagement into fusogenic protein complexes, and application of exogenous sphingosine can promote this. Because of its high diffusibility, sphingosine is most likely produced locally by a ceramidase. Five human ceramidases have been identified, of which only the neutral ceramidase, a homolog of fly Slab, has been localized to the plasma membrane. Its active center

resides on the external (noncytosolic) surface (Mao and Obeid, 2008). This implies that the newly produced sphingosine must translocate across the plasma membrane and across the cytosol to reach synaptobrevin (Figure 1, top row). Alternatively, the ceramidase may reside in the synaptic vesicle lumen (Figure 1, bottom row). This is less likely because no ceramidases were found in a quantitative analysis of synaptic vesicle constituents (Takamori et al., 2006). In addition, the positively charged sphingosine would need a facilitator to leave the luminal leaflet of the vesicle membrane (as argued above). Such facilitators have been described, for instance the Niemann-Pick type C NPC1 protein (Lloyd-Evans et al., 2008), but this protein was also not found in synaptic vesicles (Takamori et al., 2006). Finally, not only the production of sphingosine, but also of its precursor, ceramide, may be regulated locally at the active zone (not depicted). This could assist the secretion promoting effects of sphingosine by activating a phosphatase activity as Sit4/CAPP and dephosphorylation of relevant proteins at the target membrane. This proposed mechanism is analogous to the way diacylglycerol promotes the activity of a specific class of molecules (C1-domain-containing proteins), although in the case of sphingosine, the interaction between protein and lipid has not been precisely defined yet.

Many labs would probably give their annual budget for the methodology that would allow them to directly observe what is going on in the microdomains at the active zone, where synaptic vesicles, the proteins of the fusion machinery, Ca^{2+} channels, and lipid domains all reside together and where everything important with regard to secretion seems to happen. Unfortunately, methods are still lacking to observe the proteins generating the force to merge the lipid bilayers, to monitor local lipid production, and to witness the rearrangements in lipid domains, protein complexes, and their reciprocal interactions. Until that time, we have to rely on indirect evidence. One crucial direction will be to localize ceramidases, phospholipases, and DAG-lipases to define their site of action, their potential activity dependence, and to find specific and acute ways to interfere with their activity.

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Inhibitory Plasticity in Auditory Cortex

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Arguably the most important property of neuronal circuits in general, and of cortical circuits in particular, is plasticity—the ability to change in response to past experience. While many studies of plasticity emphasize changes in excitatory transmission, in this issue of *Neuron*, Galindo-Leon et al. demonstrate the important role that increased inhibition may play in shaping cortical responses to behaviorally relevant stimuli.

The cerebral cortex is plastic. The strength of the connections between neurons can change at multiple timescales, from seconds to years, and this ability is crucial for adapting animals (including humans) to their changing environment. Plasticity is strongest during early development: critical periods open and close at very young ages, determining the largescale structure of sensory cortices (Hensch, 2005). However, the cortex remains plastic even in adulthood.

Studies of plasticity in adult auditory cortex have a long history. Weinberger (reviewed in Weinberger, 2004) used